# DATA EVALUATION RECORD

1. **CHEMICAL:** Bromoxynil, octanoate
Shaughnessey Number 035302.

2. **TEST MATERIAL:** Bromoxynil Octanoate Technical; 2,6-dibromo-4-cyanophenyl octanoate; M & B Lot No. CN-51033 (20-DLM-152-1); Analytical Log No. 14542; 97.2% active ingredient; a brown solid.

3. **STUDY TYPE:** Growth and Reproduction of Aquatic Plants - Tier II. Species Tested: Anabaena flos-aquae.

4. **CITATION:** Giddings, J.M. 1990. Bromoxynil Octanoate - Toxicity to the Freshwater Bluegreen Alga Anabaena flos-aquae. Prepared by Springborn Laboratories, Inc., Wareham, Massachusetts. SLI Report #90-8-3434. SLI Study #10566.1089.6142.420. Submitted by Rhone-Poulenc Ag Company, Research Triangle Park, North Carolina. MRID Number 416060-05.

5. **REVIEWED BY:**

Kimberly Rhodes
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

**Signature:** Kimberly Rhodes
**Date:** February 11, 1991
Charles Lum 2/13/91

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

**Signature:** P. Kosalwat
**Date:** 2/12/91

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

**Signature:** Henry T. Craven
**Date:** 2/19/91

7. **CONCLUSIONS:** This study is NOT scientifically sound ~~but~~ AND does not fulfill the guideline requirements for a Tier II growth and reproduction test using a non-target aquatic plant. Due to the inconsistency of the measured concentrations, the actual exposure concentrations of this test are not known. Based on cell density, the 5-day EC50 value was determined to be >0.63 mg/L (the highest mean measured concentration tested). The 5-day NOEC value was determined to be 0.63 mg/L mean measured concentration. Therefore, Bromoxynil is not expected to exert a detrimental effect on the blue-green



Upgraded to supplemental
(See 0170117)
7 hrs

alga (Anabaena flos-aquae) when applied at application rates up to 0.375 lbs a.i./A. Based on these results a Tier III toxicity test is not required.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Organism:** The freshwater blue-green alga (Anabaena flos-aquae) used in this toxicity test was originally obtained from Carolina Biological Supply Company located in Burlington, North Carolina. The stock culture was maintained under test conditions at the testing facility.

Stock cultures were transferred to fresh medium approximately once or twice a week. The inoculum used to initiate the toxicity test with Bromoxynil was taken from a stock culture that had been transferred to fresh medium ten days before testing.

The culture medium used was Algal Assay Procedure (AAP) medium prepared with distilled deionized water and adjusted to pH 7.5 ± 0.1 with 0.1N hydrochloric acid after autoclaving. Stock cultures were grown in 125-mL glass flasks containing 50 mL of medium. The flasks were covered with stainless steel caps which permitted gas exchange.

B. **Test System:** The phytotoxicity test was conducted in an environmental chamber at a temperature of 22-26°C. The test vessels were sterile 125-mL flasks fitted with stainless steel caps which permitted gas exchange. The flasks were impartially placed on an orbital shaker set at 100 rpm. Lighting was provided continuously at an intensity of 1,500-2,200 lux at the solution surface.

The AAP medium used to prepare the exposure solutions was formulated in the same manner as the culture medium (excluding $Na_4EDTA$).

C. **Dosage:** Five-day growth and reproduction test. The nominal test concentrations of Bromoxynil based on active ingredient were 0.063, 0.13, 0.25, 0.50, and 1.0 mg/L.

D. **Design:** Based on the results of preliminary testing, a control, solvent control, and five nominal Bromoxynil concentrations (see Section 11.C) were selected for testing. The solvent control contained 0.1 mL/L of acetone which was equivalent to the concentration of solvent present in all test solutions. Each concentration and control were replicated three times.

After the test solutions were added to the flasks, an inoculum of Anabaena flos-aquae cells calculated to provide 0.3 x $10^4$ cells/mL was aseptically introduced into each flask. The inoculum volume was 970 µL per flask. At each 24-hour interval, cell counts were conducted on each replicate vessel using a hemacytometer and a compound microscope. One sample was taken from each flask for counting. One or more hemacytometer fields, each 0.1 x 0.1 cm in surface area and 0.01 cm deep and containing 0.0001 mL of culture, were examined for each sample until at least 400 cells or four fields were counted; when cell densities were less than 100 x $10^4$ cells/mL, fewer than 400 cells were present in four fields but no more than four fields were counted.

Water quality parameters (pH and conductivity) were measured at test initiation and termination. Measurements at test initiation were conducted on the test solutions remaining in the 500-mL volumetric flasks after the test flasks had been filled. At test termination, the remaining test solution in each replicate of each test concentration were composited and a portion of the composite solution was transferred to a 100-mL beaker for pH and conductivity measurement. Temperature was measured continuously with a minimum/maximum thermometer in a flask of water placed next to the test vessels. The shaking rate of the orbit shakers was recorded daily. The light intensity of the test area was measured with a light meter at test initiation and each 24-hour interval of the exposure period.

At test initiation, samples for analysis were removed from the 500-mL volumetric flasks of the test solutions and the controls and frozen for analysis. In addition, six quality control (QC) samples were prepared. The results of the analysis of these QC samples were used to judge the precision and quality control maintained during the analytical process. All solutions were

analyzed for Bromoxynil by high pressure liquid chromatography.

E. **Statistics:** Since no concentrations of Bromoxynil tested resulted in a reduction in cell density as compared to the control solutions, EC values were not calculated.

A t-test was used to compare the controls with solvent controls. Comparison of controls with solvent controls indicated no significant difference (P = 0.01) in cell density. The data from the two sets of controls were therefore pooled for analysis. Before conducting the analysis, the data were checked for normality using the Chi-Square test and for homogeneity of variance using Hartley's Test. The no-observed-effect concentration (NOEC) was determined using one-way analysis of variance and Bonferroni's Test since treatment groups had unequal numbers of replicates (i.e., control data were pooled).

12. **REPORTED RESULTS:** The mean measured concentrations of Bromoxynil were 0.029, 0.073, 0.14, 0.47, and 0.63 mg/L (Table 2, attached). The mean measured concentrations ranged from 47 to 95 percent of the nominal concentrations. Analysis of the QC samples on days 0 and 5 resulted in recoveries averaging 100 and 93%, respectively.

Cell densities determined at each observation time are presented in Table 3 (attached). All cells appeared normal. Because of the filamentous growth form of Anabaena flos-aquae, cell counts were very irregular. At test termination, the cultures were sonicated for one minute to break up chains before counting, but the numbers were still highly variable within each treatment group.

There was no reduction in cell densities at any Bromoxynil concentration over the range of concentrations tested. Furthermore, Bonferroni's Test indicated that none of the Bromoxynil treatment groups had significantly fewer cells than the pooled controls (P = 0.05). Therefore, the 120-hour EC50 was estimated to be greater than 0.63 mg/L, the highest concentration tested.

During the study, conductivity ranged from 90 to 110 µmhos/cm. At test initiation, the pH ranged from 7.1 to 7.4. At test termination, the pH ranged from 7.7 to 8.0. Continuous temperature monitoring established that the temperature ranged from 22 to 26°C during the study.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** Bromoxynil concentrations as high as 0.63 mg a.i./L do not inhibit the growth of Anabaena flos-aquae. This concentration is higher than the concentration (0.275 mg a.i./L) that would occur if Bromoxynil were applied at the maximum application rate (0.375 lb a.i./acre) to a 15-cm water column.

The study was conducted following the intent of the Good Laboratory Practice Regulations and the study conduct, raw data and final report were reviewed by Springborn Laboratories, Inc. Quality Assurance Unit. A Quality Assurance Statement was included and signed by the Quality Assurance Manager.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedure and the report were generally in accordance with the SEP and Subdivision J guidelines, except for the following deviation:

o The SEP states that the pH of the medium should be approximately 7.5. During this study, the pH ranged from 7.1 to 8.0.

B. **Statistical Analysis:** Statistical analysis was not needed since no concentration of Bromoxynil tested resulted in a reduction in cell density as compared to the controls. Therefore, the EC50 value was determined to be >0.63 mg/L (the highest mean measured concentration tested). The NOEC was determined to be 0.63 mg/L mean measured concentration.

C. **Discussion/Results:** This study is scientifically sound but does not fulfill the guideline requirements for a Tier II growth and reproduction test using a non-target aquatic plant. Measured concentrations of the test solutions at 0 hour and 120 hours are inconsistent (Table 2, attached).. Concentrations in some test levels increased from 0 hour to 120 hours, while some decreased. Initial measured concentrations of 56-67% of the nominal values might indicate the problem with the solubility of the test material. Since the water samples were not filtered before chemical analysis, some samples might have contained precipitates or particles which contributed to the inconsistency of measured concentrations observed. Therefore, the actual exposure concentrations in this test are not known.

Daily cell counts of Anabaena flos-aquae appeared to be highly variable within treatment groups. The author explained this variability to be due to clumping of the filamentous growth form of Anabaena flos-aquae and sonicated the samples prior to counting at test termination (day-5). The day-5 cell counts were still highly variable, however, each replicate of all test concentrations showed greater cell counts in comparison to the solvent control (i.e., stimulation). The mean cell counts of each test concentration ranged from 88% to 159% stimulation in comparison to the solvent control and 33% to 84% stimulation in comparison to the control. Therefore, the 5-day EC50 value of Bromoxynil for Anabaena flos-aquae was determined to be >0.63 mg/L (the highest mean measured concentration tested). The 5-day NOEC was determined to be 0.63 mg/L mean measured concentration.

Direct application of 0.375 lb a.i./acre (A) to a one acre, 0.5 feet deep pond would result in an estimated environmental concentration (EEC) of 0.275 mg a.i./L, which is lower than the estimated 5-day EC50 value of >0.63 mg/L mean measured concentration. Therefore, Bromoxynil is not expected to exert a detrimental effect on the blue-green alga (Anabaena flos-aquae) following normal application methods at rates up to 0.375 lbs a.i./A. Based on these results a Tier III toxicity test is not required.

D. **Adequacy of the Study:**

(1) **Classification:** ~~Invalid~~. Upgraded to core (See D170117)

(2) **Rationale:** Actual exposure concentration are not known.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 01-25-91.

Page_____ is not included in this copy.

Pages 7 through 9 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Shaughnessy No. 035301 | Chemical Name Bromoxynil Octanoate | Chemical Class ____ | Page ____ of ____

| Study/Species/Lab/ Accession | Chemical % a.i. | Results | Reviewer/ Date | Validati Statu |
|---|---|---|---|---|
| 14-Day Single Dose Oral $LD_{50}$ | | LD50 = mg/kg ( 95% C.L. ) Contr. Mort.(%) = | | |
| Species | ____ | Slope = # Animals/Level = Age(Days) = Sex = | ____ | ____ |
| Lab | | 14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( ) | | |
| Acc. | | Comments: | | |
| 14-Day Single Dose Oral $LD_{50}$ | | LD50 = mg/kg. ( 95% C.L. ) Contr. Mort.(%) = | | |
| Species | ____ | Slope = # Animals/Level = Age(Days) = Sex = | ____ | ____ |
| Lab | | 14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( ) | | |
| Acc. | | Comments: | | |
| 8-Day Dietary $LC_{50}$ | | LC50 = ppm ( 95% C.L. ) Contr. Mort.(%) = | | |
| Species | | Slope = # Animals/Level = Age(Days) = Sex = | | |
| Lab | ____ | 8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( ) | ____ | ____ |
| Acc. | | Comments: | | |
| 8-Day Dietary $LC_{50}$ | | LC50 = ppm ( 95% C.L. ) Contr. Mort.(%) = | | |
| Species | | Slope = # Animals/Level = Age(Days) = Sex = | | |
| Lab | ____ | 8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( ) | ____ | ____ |
| Acc. | | Comments: | | |
| 48-Hour $LC_{50}$ | | LC50 = pp__ ( 95% C.L. ) Contr. Mort.(%) = Sol. Contr. Mort.(%) = | | |
| Species | | Slope = # Animals/Level = Temperature = | | |
| Lab | ____ | 48-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( ) | ____ | ____ |
| Acc. | | Comments: | | |
| 96-Hour $LC_{50}$ | | LC50 = pp__ ( 95% C.L. ) Con. Mor(%) = Sol. Con. Mor.(%) = | | |
| Species | | Slope = # Animals/Level = Temp. = | | |
| Lab | ____ | 96-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( ) | ____ | ____ |
| Acc. | | Comments: | | |
| 120-Hour $LC_{50}$ | | EC50 = >0.63 ppm ( 95% C.L. N/A ) Con. Mort.(%) = N/A Sol. Con. Mort.(%) = N/A | K.L. 04/25/91 | Envalic |
| Species Anabaena flos-aquae | 97.2% | Slope = N/A * cells/mL = 3,000 (initial inoculum) Temp. = 24±2°C | | |
| Lab Springborn Laboratories | | 120-Hour Dose Level ppm /(% Stimulation) 0.029(101) 0.073(144) 0.14(159) 0.47(101) 0.63(103) | | |
| MRID# 416060-05 | | Comments: All responses were stimulatory relative to the solvent control. All values based on mean measured concentrations. | | |